

## Short Communication



# The detection of Pregnancy Associated Glycoproteins in the blood Serum as an Indicator of Pregnancy in Bali Cattle

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**Abstract** | Detection of early pregnancy in livestock is an important part of livestock reproductive management; therefore, choosing an accurate method for diagnosing early pregnancy is very important. This research aims to determine the potential utilization of pregnancy associated glycoproteins (PAG) circulating in the blood as a diagnostic tool for pregnancy in cows. A study was carried out on 37 Bali cows with previous experience giving birth. Blood samples were taken from the jugular vein on the 45th day after artificial insemination and stored at -20°C until PAG testing using the ELISA method. The sensitivity of the test for pregnancy detection was 0.33 ng/mL. As a control, samples from non-pregnant cows were used. The PAG concentration in pregnant Bali cows was 0.55 ng/mL, and in non-pregnant cows, it was 0.06 ng/mL. PAG concentration in pregnant cows was significantly different from that of cows that were not pregnant. The results of research on Bali cows showed that the accuracy of the PAG test in diagnosing pregnancy was 100%. Therefore, it was concluded that the circulating concentration of PAG is a suitable marker for determining pregnancy in Bali cattle. However, in order to better manage the reproductive system of Bali cows, further research is needed to increase the prediction value to a more acceptable starting point.

**Keywords** | Bali cattle, Biomarkers, ELISA, PAG, Pregnancy, Sensitivity

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## INTRODUCTION

The inability of national meat production in Indonesia has resulted in the government still importing beef from several countries. Of course, this indicates that beef production still needs to be put up to target (Sawitri et al., 2021). Efforts can be made to improve the livestock system in Indonesia, especially to achieve the target of one-

year birth intervals or to shorten the calving-conception interval by detecting early pregnancy in cows after mating (Kaya et al., 2016). Early pregnancy diagnosis is necessary after mating for early removal of non-pregnant livestock. Early pregnancy diagnosis with a high level of accuracy is very important to improve the reproductive performance of cows (Abdullah et al., 2014; Lavon et al., 2022). Early pregnancy diagnosis is very important to determine

whether the cow is pregnant so that early treatment can be carried out (Bekele et al., 2016; Prasanna Kumar et al., 2020).

Diagnosis of pregnancy, especially early pregnancy, has developed rapidly over the last few decades. Early pregnancy diagnosis is very important to minimize the time spent on cows not being pregnant (Reese et al., 2016). Several pregnancy diagnosis methods have been clinically used in cattle farming. These methods are rectal palpation, ultrasonography, biological indicators, and other early pregnancy factors. However, none of these methods are ideal for cattle. This is due to limitations in sensitivity, specificity, speed of examination, and ease of carrying out examinations (Singh, 2021; Balhara et al., 2013).

Advances in molecular techniques such as proteomics have opened up opportunities for using molecular biomarkers of early pregnancy in cows. It is thought that changes in the blood protein profile occur from estrus to implantation. Since fertilization, molecular changes have also resulted from the conceptus's development. These molecular changes may be used as specific markers of the animal's physiological stage. For this molecule to be used as a pregnancy marker. As early as feasible, the candidate molecule must identify if cows are pregnant reliably. The pregnancy-specific proteins from biological fluids are available to determine gestation (Szelényi et al., 2021).

In ruminants, glycoproteins are produced during pregnancy, which are synthesized by mono- and binucleate cells from the trophoctoderm (Shahin et al., 2014; Krebs et al., 2022). Pregnancy associated glycoproteins (PAG) appears in the mother's blood immediately after implantation and is detected throughout the pregnancy. The presence of PAG indicates the presence of a functional placenta and developing embryo (Filho et al., 2020). Although there is evidence in ruminants, the potential use of circulating PAG as a pregnancy biomarker in Bali cows has never been investigated. Therefore, developing PAG for early pregnancy diagnosis requires a comprehensive study of the PAG profile circulating in the blood serum of pregnant and non-pregnant cows. Thus, current study aimed to determine the potential utilization of PAG circulating in the blood as a diagnostic tool for pregnancy in Bali cows.

## MATERIALS AND METHODS

### ANIMAL

Thirty seven , healthy Bali cows were assigned to the study and were maintained at the Bali Cattle Breeding Centre Pulukan, Jembrana, Bali. The experimental cows were placed in a communal pen and reared in the intensive management system. All cows had normal intakes and had no

incidents of reproductive health problems. For blood sampling, 16 pregnant and 21 non-pregnant female Bali cows resulting from artificial insemination were used. Confirmation of whether a cow is pregnant or not pregnant was determined using ultrasonography. All the experimental procedures were carried out in accordance with the approval of the Animal Ethics Committee of the Faculty of Veterinary Medicine, Udayana University.

### SERUM PREPARATION

A 10 ml of Bali cow blood was taken from the jugular vein using a 10 mL vacutainer tube (BD Vacutainer, Becton, Dickinson and Company, NJ) on the 45th day after artificial insemination. Serum was obtained by centrifuging blood at 1500 rpm for 20 minutes. The serum was placed in cryotubes and stored at -20C until analysis.

### BOVINE PREGNANCY-ASSOCIATED GLYCOPROTEINS ASSAY

PAG concentration was measured using ELISA. The ELISA test uses a commercial kit (IDEXX®- Visual Rapid Pregnancy Test). The sensitivity of the test for detecting pregnancy was 0.33 ng/mL. As a control, non-pregnant cows were used. In order to reduce sample variability, the ELISA test was run with duplicates of each sample from pregnant cows approximately 45 days after pregnancy. In all experimental cows, an ultrasound examination was carried out to confirm pregnancy status on day 45 (DG45), and this ultrasound examination was considered the gold standard reference.

### STATISTICAL ANALYSIS:

The accuracy of PAG concentration in serum was determined by analyzing the examination results with the actual reproductive status of pregnant or non-pregnant cows. Differences in PAG level concentrations in pregnant and non-pregnant cows were analyzed using the T-test. The results of examining PAG concentrations were presented as mean  $\pm$  SEM, and differences were considered significant if  $P < 0.05$ .

## RESULTS

Among the 37 blood samples examined using the PAG-ELISA test, a total of 16 cows were identified as pregnant, and 21 were not. No false positives were observed in the blood serum examination. The results of examinations using the PAG-ELISA test showed that serum samples identified as pregnant showed an average titer of  $0.552 \pm 0.242$ , and cows identified as not pregnant showed an average titer of  $0.067 \pm 0.103$  (Table 1). The accuracy of the examination results was found 100% in Bali cows. The statistical analysis results showed a significant difference in circulating PAG concentrations in the blood serum

of pregnant and non-pregnant Bali cows ( $P < 0.001$ ). The results of this PAG examination can be used to validate pregnancy in Bali cattle.

**Table 1:** The average concentration of PAGs found in the blood serum of Bali cows.

| No. | Sample            | Average concentration ( $\mu\text{g/ml}$ ) |
|-----|-------------------|--------------------------------------------|
| 1   | Pregnant cows     | $0.552 \pm 0.242^a$                        |
| 2   | Non Pregnant cows | $0.067 \pm 0.103^b$                        |

Different superscript letters indicate significant differences ( $p < 0.05$ )

## DISCUSSION

Successful pregnancy is a significant problem for the economic income of cattle breeders. Detection of early pregnancy in livestock and prediction of early pregnancy failure is beneficial in influencing livestock management decisions (Pohler et al., 2020; Barbato et al., 2022). Diagnosis of early pregnancy is an important tool in livestock species and has been performed traditionally via transrectal palpation or ultrasound. However, due to limitations in skilled technicians and their height, the demand for accurate pregnancy diagnosis has become increasingly important (Khan et al., 2020). Pregnancy diagnosis is an important part of successful reproductive management (Delhez et al., 2020) and early identification of non-pregnancy in female cattle. Knowing early pregnancy will reduce the mating interval, give birth, calving intervals and early re-breeding (Filho et al., 2020).

Glycoproteins are protein pregnancy markers that are extensively glycosylated secretory proteins of trophoblast cells of the fetus and released in the maternal bloodstream. This glycoprotein can be seen circulating in the dam's blood circulation for 30 days in all pregnant cows (Krebs et al., 2022) and will continue to increase until it reaches its peak about one week before calving. These proteins secreted by the placenta are associated with conception and are called pregnancy-associated glycoproteins (PAGs). These diagnostic tools identify pregnant or non-pregnant animals and predict early pregnancy failure (Barbato et al., 2022).

The results of this study show that a pregnancy test using circulating proteins in Bali cow serum can detect pregnancy status accurately. The PAG assay produced several true positives (16/16) and measured true negatives (21/21), and the PAG test method was accurate for detecting pregnancy in Bali cows. This glycoprotein provides accurate results for detecting pregnancy. This pregnancy kit can consistently detect the presence of PAG in the serum of pregnant and non-pregnant Bali cows. Testing with the PAG commercial kit can diagnose pregnancy in cows accurately and is available in the market for blood and milk (Filho et al.,

2020).

The sensitivity of test PAG-ELISA was reported to be 100% (Ghaidan et al., 2019; Palhão et al., 2019), and Northrop et al. (2019) reported this assay was 97%. In Bali cattle, it was found that there was a relationship between PAG circulating in serum during pregnancy with accuracy reaching 100%. This research result confirmed that circulating PAG concentrations are elevated in pregnant in day 45 compared to non-pregnant Bali cattle. This research aligns with the findings by Commun et al. (2016), who stated that the sensitivity of using PAG as a pregnancy marker reaches 100%. PAG testing is the best technique for detecting pregnancy in cows, especially after one month of pregnancy (Ghaidan et al., 2019). PAG-based ELISA test as a good method for pregnancy diagnosis because quite specific and sensitive for diagnosing pregnancy in cattle farms (Kaya et al., 2016).

After successful conception, PAG may be detected at three weeks after conception. Concentrate PAG slowly increases until just before birth; the sensitivity and accuracy of PAG as pregnancy markers increase according to the pregnancy age. Early pregnancy diagnosis in cows is important, especially in management and economics. The potential of any pregnancy screening method must be compared with the gold standard, namely transrectal ultrasound (Palhão et al., 2019).

Pregnancy diagnosis based on the 45th day PAG concentration in Bali cows that has been tested is comparable to the accuracy of using ultrasound, including the potential of this PAG in determining whether Bali cows are not pregnant. The role of early PAG concentration for pregnancy checks in Bali cows currently seems could not be carried out because it is due to the sampling problem that ultrasound examinations only be carried out on the 45th day.

Balinese cattle are germplasm of Indonesia, resulting from direct domestication from bulls, which still live in the Baluran National Park area, East Java (Martoyo, 2012). Bali cattle have powerful energy, and the presence of horns on cows, accompanied by their wild temperament, requires special techniques or skills in handling cattle, especially during pregnancy checks. This often makes it impossible to carry out pregnancy checks at an early age. Therefore, this invasive examination gives hope for early pregnancy checks in Bali cows.

Results showed that PAG levels cycled in only pregnant animals. Therefore, this PAG can be used to validate the pregnancy of Bali cows. Based on the current study findings, the ELISA kit developed for bovine PAG detection is suitable for accurate pregnancy diagnosis in Bali cows at



## CONCLUSION

Diagnosis of early pregnancy in Bali cows using PAG concentration circulating in blood serum has the potential to be developed as pregnancy markers in Bali cows. PAG concentration in pregnant Bali cow is significantly different from that of cows that are not pregnant. Circulating PAG concentration may be a suitable marker for determining pregnancy in Bali cows; however, in order to better reproductive management of Bali cows, further research is needed to increase the prediction value to a more acceptable starting point.

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## CONFLICT OF INTEREST

No conflict of interest.

## NOVELTY STATEMENT

This is the first report on using pregnancy-associated glycoproteins for pregnancy detection in Bali cattle.

## AUTHOR CONTRIBUTIONS

IKP and INS: conceptualization, supervision, writing, review and editing; NNT: writing original draft; NWP, MRK, PBS: data curation; IWS, AAGOD, PDJ and IWNFG, writing original draft preparation. All authors have read and agreed to the published version of the manuscript.

## REFERENCES

- Abdullah M, Mohanty TK, Kumaresan A, Mohanty AK, Madkar AR, Baithalu RK, Bhakat M (2014). Early pregnancy diagnosis in dairy cattle: economic importance and accuracy of ultrasonography. *Adv. Anim. Vet. Sci.* 2 (8): 464-467. <http://dx.doi.org/10.14737/journal.aavs/2014/2.8.464.467>.
- Balhara AK, Gupta M, Singh S, Mohanty AK, Singh I. (2013). Early pregnancy diagnosis in bovines: current status and future directions. *Scient. World J.* 5:958540. <https://doi.org/10.1155/2013/958540>. PMID: 24382949;

- Barbato O, Menchetti L, Brecchia G, Barile VL. Using Pregnancy-Associated Glycoproteins (PAGs) to Improve Reproductive Management: From Dairy Cows to Other Dairy Livestock (2022). *Animals*.12(16):2033. <https://doi.org/10.3390/ani12162033>.
- Bekele N, Addis M, Abdela N, Ahmed WH (2016). Pregnancy Diagnosis in Cattle for Fertility Management: A Review. *Global Vet.* 16 (4): 355-364. <https://doi.org/10.5829/idosi.gv.2016.16.04.103136>.
- Commun L, Velek K, Barbry JB, Pun S, Rice A, Mestek A, Egli C, Leterme S (2016). Detection of pregnancy-associated glycoproteins in milk and blood as a test for early pregnancy in dairy cows. *J. Vet. Diagnost. Investigat.* 28(3) 207-213. <https://doi.org/10.1177/1040638716632815>.
- Delhez P, Ho PN, Gengler N, Soyeurt H, Pryce JE (2020). Diagnosing the pregnancy status of dairy cows: How useful is milk mid-infrared spectroscopy?, *J. Dairy Sci.*, 103(4): 3264-3274. <https://doi.org/10.3168/jds.2019-17473>.
- Filho RVO, Franco GA, Reese ST, Dantas FG, Fontes PLP, Cooke RF, Rhinehart JD, Thompson KW, Pohler KG (2020). Using pregnancy-associated glycoproteins (PAG) for pregnancy detection at day 24 of gestation in beef cattle, *Theriogenology*,141,2020,128-133. <https://doi.org/10.1016/j.theriogenology.2019.09.014>
- Ghaidan MT, Dana OI, Dyary HO (2019). Accuracy of bovine pregnancy-associated glycoproteins (bPAGs) in the diagnosis of pregnancy: A comparative study of three pregnancy diagnostic methods. *Polish J. Vet. Sci.* 22 (4): 769-775. <https://doi.org/10.24425/pjvs.2019.13140>.
- Kaya MS, Kose M, Bozkaya F, Mutlu H, Ucar EK, Atli MO (2016). "Early pregnancy diagnosis using a commercial ELISA test based on pregnancy-associated glycoproteins in Holstein-Friesian heifers and lactating cows," *Turk. J. Vet. Anim. Sci.* 40 (6) Article 3. <https://doi.org/10.3906/vet-1602-41>
- Khan D, Khan H, Ahmad N, Tunio MT, Tahir M, Khan MS, Khan RU (2020). Early Pregnancy Diagnosis using PregnancyAssociated Glycoproteins in the Serum of Pregnant Ruminants. *Pakistan J. Zool.* 52(2): 785-788, DOI: <http://dx.doi.org/10.17582/journal.pjz/20190527080541>
- Krebs T, Kilic I, Neuenroth L, Wasselin T, Ninov M, Tetens J, et al. (2022) A multiplexed parallel reaction monitoring assay to monitor bovine pregnancy-associated glycoproteins throughout pregnancy and after gestation. *PLoS ONE.* 17(9): e0271057. <https://doi.org/10.1371/journal.pone.0271057>
- Lavon Y, Friedman S, Shwimmer A, Falk R (2022). Performing Early Pregnancy Tests in Milk and Their Effect on Cow Welfare and Reproductive Performance Compared to Rectal Pregnancy Tests 40 to 45 Days Post Insemination. *Dairy.* 3(3):465-473. <https://doi.org/10.3390/dairy3030034>.
- Martojo H (2012). Indigenous Bali Cattle is Most Suitable for Sustainable Small Farming in Indonesia. *Reprod. Dom. Anim.* 47 (Suppl. 1), 10-14. <https://doi.org/10.1111/j.1439-0531.2011.01958.x>
- Northrop EJ, Rich JJJ, Rhoades JR, Perry GA (2019) Comparison of two bovine serum pregnancy tests in detection of artificial insemination pregnancies and pregnancy loss in beef cattle. *PLoS ONE.* 14(1): e0211179. <https://doi.org/10.1371/journal.pone.0211179>.
- Palh o MP, Guimar es CRB, Lima JFJFM, Mendon a MR, Fernandes CAC, Neves JP, Garcia JAD, Gioso MM, Miglino MA, Viana JHM (2019). Efficacy and limitations of different

- approaches to anticipate the diagnosis of pregnancy in cattle. *Arq. Bras. Med. Vet. Zootec.* 71 (6):1909-1916. <https://doi.org/10.1590/1678-4162-10698>.
- Pohler KG, Reese ST, Franco GA, Oliveira Filho RV, Paiva R, Fernandez L, Melo G, Vasconcelos JLM, Cooke R, Poole RK (2020). New approaches to diagnose and target reproductive failure in cattle. *Anim. Reprod.* 17(3):e20200057. <https://doi.org/10.1590/1984-3143-AR2020-0057>
- Prasanna Kumar C, Chatrpathy K, Manjula V, Thangadurai N (2020). A Novel Technique for Early Pregnancy Detection of Dairy Cattle. *Int. J. Innovat. Sci. Engineer. Technol.*, 8 (2), February 2021
- Reese ST, Pereira MC, Vasconcelos JLM, Smith MF, Green JA, Geary TW, Peres RFG, Perry GA, Pohler KG (2016). Markers of pregnancy: how early can we detect pregnancies in cattle using pregnancy-associated glycoproteins (PAGs) and microRNAs?. *Anim. Reprod.* 13 (3): 200-208, <https://doi.org/10.21451/1984-3143-AR878>.
- Sawitri NM, Trilaksana IGNB and Puja IK (2021). Evaluation of Bali cattle semen quality during cryopreservation with coconut water-based extenders. *Int. J. Vet. Sci.* 10(4): 329-334. <https://doi.org/10.47278/journal.ijvs/2021.064>.
- Szelényi Z, Szenci O, Kovács L, Garcia-Isperto I (2021). Practical Aspects of Twin Pregnancy Diagnosis in Cattle. *Animals (Basel)*.8;11(4):1061. <https://doi.org/10.3390/ani11041061>.
- Shahin M, Friedrich M, Gauly M, Holtz W (2014). Pregnancy-associated glycoprotein (PAG) profile of Holstein-Friesian cows as compared to dual-purpose and beef cows. *Reprod. Domest. Anim.* 49(4):618-620. <https://doi.org/10.1111/rda.12336>.
- Singh SP, Natesan R, Sharma N , Goel AK, Singh MK, Kharche SD (2021). Assessment of pregnancy-associated glycoprotein profile in milk for early pregnancy diagnosis in goats. *Anim. Biosc.* 34(1):26-35. <https://doi.org/10.5713/ajas.19.0399>